

INTRODUCTORY NOTES ON A BIBLIOGRAPHY

by

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October 1978

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My first paper having to do with lipids was published in 1954 (5). It demonstrated that ligation of the bile duct caused a great increase in hepatic cholesterol synthesis. The work was done with Ivan Frantz in his laboratories at the Massachusetts General Hospital in the period 1951-1953. We also discovered that drainage of the bile had the same effect, but could not exclude partial obstruction by the drainage tube as the more proximate cause. We did not report it, and thus missed the opportunity "to discover" the feedback control of bile on hepatic cholesterol synthesis.

The techniques and sub-disciplines to which a young scientist is early exposed often set the paradigm which he follows for the rest of his career. So it was with water-insoluble substances and me. When I left Boston, I joined Anfinsen and Horning who had found a mitochondrial system for oxidation of cholesterol (15). Some new oxidation products were recoverable (9) and later identified as 25- and 26-hydroxycholesterol (12). This work was done in Bethesda where I had taken up a two-year appointment at the National Heart Institute. Two of my fellow clinical associates (in the "opening class" at NIH) were Richard Havel and Robert Gordon. We all were in the laboratory of Christian B. Anfinsen where my early interest in cholesterol metabolism began to swing to the lipoproteins in which this sterol made its way around the plasma. I had brought some knowledge of radioisotopes to Bethesda, skills which then were not nearly as commonplace as now. Havel, who was becoming an expert in using preparative ultracentrifuge, and I elected to combine techniques. We collected C<sup>14</sup>-palmitate-labeled chylomicrons from

dog lymph, reinjected them and reported the first observations of how labeled chylomicrons are removed from circulation (11).

We observed (23) the early appearance of some of this labeled fat in a plasma fraction which Gordon and Dole at the Rockefeller Institute were simultaneously resurrecting from vague references in older literature. Gordon called this fraction "unesterified fatty acid" (UFA) and Dole preferred the term "non-esterified fatty acids" (NEFA). Their observations suggested a very rapid turnover of these "free fatty acids" (FFA), the compromise term by which they came to be called. Gordon and I made the first direct measurements of plasma turnover rates of FFA in 1957 in animals and man (16 & 25).

Two papers published in 1957 and 1958 illustrate how easy it was to become well known in the 50's. Anfinsen received more invitations to speak and to write on lipoproteins than he could tend to. I took one to perform in New Orleans and began to tinker with new modes of describing and depicting the relationship between plasma lipids and lipoproteins (14). Thus began a series of presentations to physicians--with the requirement for yearly Spring showing of the latest finding and new slides--that was still going strong in 1974 when interrupted by my assumption of the Presidency of the Institute of Medicine, NAS.

Simultaneous with the greater medical interest in lipoproteins came a new awareness of lipid transport on the part of physiologists and biologists. Bob Gordon and I took another of Chris' defaulted invitations and turned it into the first serious review of the biological aspects of plasma lipoproteins--this was one of the better selling pieces in Physiological Reviews (26) for some time and helped us become known as specialists in those abstruse subjects

like FFA, the protein "envelopes" or "apolipoproteins" that solubilized these fat particles, and other phenomena that were viewed suspiciously by many chemists. Some 8 years later I described plasma lipoproteins to the German physiological chemists at their Spring meeting in Mosbach (60). Willi Stoffel, then a young docent in Klenk's department in Cologne, said to me after the lecture, "You did well, but they don't believe in what you are describing."

It would be too much historical reconstruction to pretend that, stung by the disbelief of the highly chemically oriented Germans, I then dedicated my career to proving that apolipoproteins were specific, i.e., not random companions of water-insoluble substances touring about in plasma. Nevertheless, the number of apolipoproteins, their specificity, and precise functions were the thread which connected all of my later work. This includes the typing of hypolipoproteinemia, of which more later. Typing, too, had as its purpose the better segregation of human mutants; when a defective gene fails to produce a functioning apoprotein, a unique opportunity to understand the function and importance of that protein may present itself. (At this writing, such mutants as abetalipoproteinemia, Tangier disease, apo-C-II deficiency, and, perhaps, apo E in type 3 hyperlipoproteinemia, are cases in point.)

One of the earliest to look for apoproteins was Marty Rodbell at NIH, who found several different amino-terminal groups in the proteins isolated with lipoproteins of different density. In the late 50's, I joined Rodbell in searching for information about apoproteins in chylomicrons. We separated lipoproteins on great slabs of urea-loaded starch. The several

different protein bands we found in dog and human chylomicrons (27, 28) we designated as "proteins A, B, and C," the forerunner of the current code for naming lipoproteins that was proposed by Alaupovic (1972).

At this same time, Stanbury, Wyngaarden, and I had met in Atlantic City and decided to write and to edit a volume containing the clinical and biochemical descriptions of the many "inborn errors" described since Garrod's classic description. Four editions of The Metabolic Basis of Inherited Diseases would appear between 1960 and 1978. Through the fourth edition of this succes d'estime, I would be primarily responsible for writing eighteen chapters, each a lengthy review, usually completely rewritten and restructured with each edition (30-33, 50-54, 130-135, 197-199). So busy were we at the times when this book was in preparation that I often placed in the chapters new data which never were separately published--a form of communication which obscures discovery, but worse, frustrates normal scientific review and criticism.

The decision to embark on this book and the requirements its revisions imposed had important effects on my work. The most significant of these was my intention to convert the Hyperlipidemias (I had to campaign long and hard for this term because hyperlipemia had much earlier been preempted to describe milky plasma or gross hypertriglyceridemia) to Hyperlipoproteinemias. I went to see Thannhauser, then the doyen of these diseases, in 1960 to seek to convert him to this approach. He was old and ill and not convertible. I still have a copy of the review of our first edition, in which Nepomuk Zöllner, a devout disciple of Thannhauser's, lamented our neglect of the European master's precepts, ". . . eine durch die Jugend der Autoren zu erklärenden Unkenntnis der alten Literature erkennen lassen." 2/

When the second edition came due, the need to improve on the state of things as they were in Thannhauser's time was more urgent; and Robert Lees and I were in the process of constructing the typing system for hypolipoproteinemia ("types I through V") that was fated to run through the rest of my work like a dominant and highly popular theme which the composer grow dreadfully tired of hearing.

In the earlier editions of MBID I had to write the definitive reviews of the sphingolipidoses. The "authorities" in those days tended to be either clinicians, neuropathologists, or chemists, and rarely were hybrid enough to cope with the full treatment we demanded for MBID. I pursued them through stacks of case reports and other literature, in numerous different languages, and eventually became familiar with a great variety of esoterica, especially neurology, ophthalmology, and the chemistry of sphingolipids. This allowed me to move in quite unusual circles for a board-certified internist (40, 66, 69, 80, 88, 100). It also gave me a special perspective from which to cope with patients with atypical lipidoses. For example, when the boy who would become case No. 1 of "Tangier disease" was referred to me as an example of "Niemann-Pick disease," it was not difficult to see that something was amiss. I was simply lucky, too, to have been well-equipped to pursue this new disease over the frontiers into lipoprotein-land where it really belonged. Our discovery of Cholesteryl Ester Storage Disease came about in the same way (135), this child having been referred to me as an example of Familial Hypercholesterolemia.

The sphingolipidoses began around 1960 to undergo a revolutionary transformation to "lysosomal hydrolase deficiencies." With this new illumination provided by DeDuve, Hers, Brady, O'Brien, and many others, the syndromes

became extraordinarily complex. Keeping up with the literature and following an increasing number of referred patients became a heavy distraction, and adaptation of the necessary biochemical techniques became a luxury that a laboratory dedicated primarily to lipoproteins could not afford. New specialists in these diseases emerged who, having discovered where the biochemical errors lay, also began to lighten the "lipidosis load" for the later editions of MBID. It is amusing to observe the few traces of our primitive nosology still visible on the sphingolipidoses. The "types 1, 2, and 3" we proposed for Gaucher's disease are still in contemporary use (133). And we had minor roles to play in Brady's important discovery of the first lysosomal enzyme deficiency (61, 101).

#### The Long Ride to Tangier

The September day in 1960 when Paul Altrocchi and I went to Tangier Island in the Chesapeake Bay opened one of the most interesting chapters in my life. I have recounted the story elsewhere (184) of Teddy Laird, the sight of the bright-orange and grotesquely enlarged tonsils in his sister Elaine's throat, and the assistance of their remarkable mother, Mrs. Peggy Laird, in exploring the genetics of the disorder which we decided to call "Tangier disease." The romantic quality of this name, conjuring up thoughts of the Casbah and medinas of Morocco, will likely cause it to survive the more accurate terms of "Familial HDL Deficiency" (15) or "Analphalipoproteinemia" (128).

In keeping with the catholicity of my interests at the time, I first described "Tangier disease" in 1961 at a symposium on cerebral sphingolipidoses, attended by people who were more interested in Tay Sachs disease and barely

conscious of tonsils (38). Altrocchi and I later discussed the disease with some colleagues at an NIH clinical conference arranged for my debut as Clinical Director of the National Heart Institute (36).

Up to this writing, Tangier disease has been mentioned or featured in 34 subsequent publications of mine. Ten of these papers contain new substantive information. Each adds one or more tiles to a steadily expanding and much larger mosaic having as its central theme the mystery of why high density lipoproteins (HDL) are found in the plasma. In an early one of these 10 papers (41), the inheritance of Tangier disease was worked out from HDL concentrations in the plasmas of scores of subjects on Tangier Island. In this project, I had the invaluable help of Mrs. Netti Crockett Pruitt, the retired school teacher on the island. She alone had records of the genealogy of the population, the preacher having destroyed the written records kept in the church. "Miz Nettie's" records were all in her head. The complex blood lines emerging from our interviews long stood in my old office like a wiring diagram. As we recognized then (41), the disease was an autosomal recessive one, but the trait was not clearly segregated by the ultracentrifuge. Two obligate heterozygotes were "normal" with the methods available. Some of the new techniques for immuno-assay of apoprotein A-1 have now been used by Assmann and by Herbert to detect accurately the heterozygote "carrier" of Tangier disease.

The second (45), third (51), and fourth (68) affected families were described in later publications; none of the subsequent families have any known relationship to the Tangier Island population. There are now about 25 known cases. This is about as many as there are cases of abetalipoproteinemia,

the disorder involving absence of the other major lipoprotein family (containing apoprotein B); it was discovered a year earlier than Tangier disease.

By 1972, enough had been learned about apoproteins to attempt a better characterization of the HDL defect in Tangier disease. The first examination of Laird plasma revealed some immunochemical reaction with antiserums prepared against HDL. They resembled some reactions later obtained by Levy in normal HDL subjected to physical or chemical manipulation (44). More than 5 more years passed before the latter reactions were explained by the presence of several HDL apoproteins. Sam Lux in our laboratory led the tedious work to show that the primary gene product most probably affected by the Tangier mutation was apolipoprotein apo A-I (144). Very small amounts of it were present in the homozygote chosen (the older Lo. sibling). Apo A-II was also deficient but less so, and the usual A-I/A-II ratio was reversed. From the findings of Lux et al., we postulated some failure in apo A-I binding or synthesis as the most likely defect. Recently, Assmann, now back in Germany and working on German examples of this mutation, has compiled evidence supporting this.

It was in 1976-77 that we were first able to isolate two distinctive abnormal lipoproteins from huge quantities of Tangier disease plasma. One we called the "A-II particles." It is smaller than normal HDL lipoproteins and contains only A-II (195). Such particles possibly are present in normal plasma, but it would not be detectable because of the great mass of "usual" HDL containing both A-I, A-II, and the C-apoproteins--of which we shall have more to say later. The second abnormal lipoprotein in Tangier plasma we have

identified as remnants of chylomicrons (195, 201, 204, 207). Thus, our latest publication (207) seems to confirm the hypothesis advanced in the first reports of Tangier disease (38, 51) that the massive storage of cholesteryl esters, which is a feature of the disease, is due to deposition of unstable chylomicrons. One function of HDL now appears to be stabilization of triglyceride-rich particles as they are delipidated in the bloodstream. HDL thus also determine the lipid content, especially the amount of triglycerides, in VLDL and LDL.

The tenth in the "selected" list from our Tangier reports is the lengthy study Victor Ferrans and I made of all the pathologic findings in Tangier disease up to 1975 (174). Our aim was to examine all the histologic explanations to be sure we had missed no clues to many of the other unanswered questions about HDL. Among them is the possible role of HDL in mobilizing cholesterol in peripheral cells for transport to the liver. This potential role has loomed larger in recent years. One reason is the demonstration by Goldstein and Brown that LDL is taken up peripherally and "destroyed," leaving a requirement for removal of a steady influx of sterol in addition to that synthesized locally. A second reason is the revival of older correlations between HDL concentrations and "protection" from premature coronary artery disease. Yet, patients with Tangier disease do not seem to have more heart disease or small vessel abnormalities (174), a paradox remaining to be explained. Another feature of the disease is a peculiarly cyclical peripheral neuropathy (68). We have found lipid deposits in the Schwann cells of the nerve sheaths in a few skin biopsies. This confirms histologic findings first made by Haas and co-workers in examination of the then oldest patient with the disease, who was shown to

me at Grand Rounds in Wellington, New Zealand, when I was on a lecture tour there in 1967. An even older patient, with the same remarkable degree of neuropathy, has now been followed at the Mayo Clinic (240). One intriguing mystery is the unique proclivity of the tonsils to collect cholesteryl esters. It makes Tangier disease the only lipoprotein defect that can be diagnosed by a look in the mouth. I think one of the most creative explanations was advanced by Dr. Donald Small, who suggested that the slightly lower temperature in the pharynx was just below that critical for maintaining the cholesteryl esters as liquid crystals. (In my Jimenez Diaz Lecture in 1973, I suggested that if we learned why we have HDL we might discover why we have tonsils)(184).

### The Typing System

Returning to the bibliography at the point of digression into Tangier disease, I see the two references in 1965 (46 and 47) which initiated the system for typing hyperlipoproteinemia. Robert S. Lees came to work with me in 1964. He brought with him from Boston a modification of paper electrophoresis which helped resolve plasma lipoproteins into more distinct bands than were described in the previous reports of paper electrophoresis for lipoproteins. The useful effect had been achieved by adding albumin to the usual barbiturate buffer. I was then--and remain--more interested in the quantification achievable by the preparative ultracentrifuge. I had learned the technique from Havel, from whom I had also inherited a few patients with severe hyperlipidemia. Their numbers and problems were growing, and the state of knowledge about them was chaotic. They fell into two lumpy groups: "essential (familial) hypercholesterolemia" and "essential hyperlipidemia," which I once referred to as "the sanctuary of the undifferentiated."

Due to demands imposed by need to revise the MBID, and my belief that hyperlipidemia due to mutation of single genes should be easier to segregate, we were seeing more and more familial cases in the clinic. We became adroit dermatologists and even dared name a xanthoma or two, viz., "tuberoeruptive xanthomas." Without much encouragement, Lees "ran out" the plasmas of all these patients and their relatives along with our other analyses. Rather quickly it began to appear as though electrophoretic patterns, if accompanied by quantification like that available in the preparative ultracentrifuge technique of Havel, Eder, and Bragdon, might give us an increased resolution of these abnormalities. Indeed a kind of useful shorthand was possible. Often given to Linnaean impulses, I began to call out to Lees, as we reviewed the strips, "This is Type 1, these will aggregate as Type 2, etc."

The system was designed to correspond to the order of lipoprotein bands on the strips so that it might be more easily remembered. Thus "Type I" was for the massive chylomicronemia at the origin of the strip, "Type II" for an increase in the beta lipoproteins (LDL) which came next, etc. A band running faster than beta, called the pre-beta band (a term first used by Elspeth Smith in Britain), had been a subject of interest to us from the first. We published some experiments indicating that this band was equivalent to very low density lipoproteins (VLDL). By feeding radically different diets to abnormal subjects we also showed that the endogenous (pre-beta rich) hypertriglyceridemia was very different from the exogenous kind characterized by its chylomicron band at the origin (47).

I had agreed to give a lecture at the American Heart Association meeting in the spring of 1965, and so we made up some slides incorporating this new

approach. The pretty oil-red-O stained strips were easily memorizable as five patterns. More importantly, each pattern had associated with it some distinctive clinical differences, xanthomas, and different responses to the available therapies. Physicians were becoming steadily more aware of the puzzling heterogeneity of patients with an elevated cholesterol or triglyceride concentration in plasma, and the emphasis on lipids as a "coronary risk factor" was constantly increasing. In response to requests for a summary of the lecture by the journal *Circulation*, the editorial entitled "Phenotyping of Hyperlipoproteinemia" appeared in 1965 (46). By this time the second edition of the MBID was due, and Lees and I decided to re-assemble the partly differentiated "essential hyperlipidemias" into the still sketchy but promising new matrix or typing system based on different lipoprotein patterns in "familial hyperlipoproteinemia" (50).

A footnote added in galley to that chapter (50) indicates growing awareness of a unique syndrome that would shortly require "types 2 and 3" to be redefined. This revision would come about partly because of the collaboration of another associate, Robert I. Levy. He began his work with me on HDL and on the pre-beta lipoproteins (57). Gradually he gave increasing attention to the quantitation of plasma lipoproteins for clinical purposes. For example, we had to be sure we were quantifying all of the  $\beta$ -migrating low density lipoproteins. Comparison of the results with ultracentrifuge and strips led to the discovery that some beta-lipoproteins were of lower density than normal. These occurred in a particular group of patients who often had yellow deposits in their palms, peripheral vascular disease, and other clinical peculiarities. I first suggested we might call this "floating beta disease."

(One day we would discover "sinking pre-beta," too.) The patients with "floating beta" became "Type III hyperlipoproteinemia." As is noted in our reports, the chemical and some of the clinical features of "Type III" (81) appear in several patients with "xanthoma tuberosum" in a paper by Gofman and co-workers in the early 50's.

In 1964, Joseph Garland, then editor of the New England Journal of Medicine, had exacted a promise from me of a Medical Progress article on lipoproteins. Postponing it repeatedly while we collected the necessary normal control data and worked out the "bugs" in the new classification system, I decided at last to make a "heroic" effort to keep my commitment. Armed with pads of foolscap and a pencil sharpener, I went to a Federation meeting in Atlantic City, but pledged not to leave the motel room, except to eat, until a first draft had been hammered out. Five days later, red-eyed and unshaven, I emerged with the incubus. Always being a 7-draft man, there was much yet to do, but Lees and Levy handed me data and bravely defended the designs I was demanding of the artists for illustrating the scheme. The review appeared in five installments in 1967 (63). I convinced Bob Berliner (then Scientific Director of NHI) he should allow us to buy an unheard-of 10,000 reprints. The "market" proved brisk enough to eliminate the inventory in a reasonable time. Citation Index has recently been counting and this review appears among its list of the several hundred "most-cited papers." <sup>3/</sup> This alone is a poor measure of scientific excellence, of course. If it were, Levy, Jones, Bonnell, and I would be the greatest scientists ever; for the diet books we put together to meet requests for advice from physicians sensitized to the types of hyperlipoproteinemia (104, 152) passed the 7 million(!) mark in distribution in 1978.

The typing system began to be very widely used after the review appeared. When I visited Australia and New Zealand, and much of Europe in the late sixties, I found clinics churning out "types" as though there might be a life-saving quality to them. In 1972, the WHO gave the system a kind of official status (116). In this report types "IIa" and "IIb" appear for the first time, lending dignity to an informal "improvement" we were already using in Bethesda.

Typing was, and remains, a very useful shorthand. It consisted of both a re-ordering of information collected earlier along with new information arising during its development. There came, in turn, need for further simplification. The types were shown to be derivable by estimations (138, 140) or techniques which do not include the ultracentrifuge (151). Typing still retains a capacity for helping the physician choose the most effective therapy, this varying with the kind of hyperlipoproteinemia (66, 73, 79, 85, 86, 95, 106, 109, 126, 146, 147, 150, 161). Above all, typing made physicians aware of lipoproteins and gave them a better window frame through which to view lipid transport and metabolism as it might affect their patients' cholesterol levels.

On the other hand, our early use of the word "phenotyping" by patterns (46), and insufficient attention to our early warnings (63), created a widespread problem of confusing a lipoprotein pattern with a single disease and, particularly, as a marker of a specific mutation. Sometimes my own colleagues failed to keep these injunctions and I still feel compelled to emphasize the difference (198). Moreover, many physicians and clinical laboratories came to believe that "lipoprotein phenotyping" was necessary whenever a patient

was routinely seen, and on the tenth anniversary of the introduction of this particular system, it was necessary to urge this routine practice be stopped (176). It was my earlier expectation that even the nomenclature would be dead and gone, replaced by more specific terminology. Actually, better terms have not yet appeared for the curious disturbances called for "type 3 or 5" hyperlipoproteinemia, and there still survives clinical and experimental usefulness for the "type" system and methodologies. Interestingly, in the Soviet Union this month, I observed "typing" activities in Leningrad proceeding with a fervor reminiscent of our "golden days." As an aside, we may note that medical students were exposed to lipoprotein "types" in the 70's only if they read one of the two rival textbooks of medicine. Havel wrote the section on lipids in Beeson's Textbook of Medicine and studiously avoided references to lipoprotein patterns by numbers. In Harrison's Textbook, I had a different orientation (107, 148, 192).

It would be foolish to deny that "typing" did not have a marked effect on how our laboratory/clinic in Bethesda was organized and how we pursued the numerous problems with which its members were simultaneously engaged. The steady stream of patients enriched the most fundamental and non-clinical work. The collection of data from over 1,000 well-classified patients made possible several studies that will probably not be repeated again at single laboratories. ("Single" is emphasized since there are now 12 Lipid Research Clinics in the U.S., Canada, Israel, several in Russia and elsewhere whose activities were originally patterned after our laboratory as a model. <sup>4/</sup> The very large Bethesda studies included extensive analyses of lipoproteins and certain clinical features of patients with familial hypercholesterolemia,

in which Peter Kwiterovich and Neil Stone played important roles (157, 167, 168). There were also reports of numerous special morphologic changes accompanying different lipoprotein patterns reported in collaboration with William Roberts and Victor Ferrans (123, 156, 177, 178).

Given that familial hyperlipidemia was first observed around the turn of the century and the subject of a large literature before we began, it is reasonable to ask the number of absolutely new diseases or mutations detected by this activity of ours between 1965 and 1975. Three new syndromes of genetic hyperlipoproteinemia were established. Ours was the first proof of the presence of familial endogenous hypertriglyceridemia (63, 198). The same is true of familial type 5 hyperlipoproteinemia (63, 198), although more proof of specific genetic defect is needed here. Probably the most interesting discovery was technically a confirmation of the several patients described earlier by Gofman; yet it was in Bethesda where type 3 hyperlipoproteinemia was first clearly separated from among the mass of hyperlipidemics and shown to be inheritable. This first came into focus in the New England Journal of Medicine review of 1967 (63), and was the subject of quite a few subsequent papers, including two early ones dealing with the possible defect (123, 156), and two relatively recent ones in which we re-examined the initial hypotheses, including means of detecting the disorder (177, 178). These last were the knell for old "floating beta" as a marker for "type 3" (I had had enough of roman numerals by 1972 and went arabic to the dismay of some of my proteges) (138). Type 3 has recently become the subject of interest in regard to apolipoprotein E, and will some day prove to be extremely instructive in regard to the metabolism of the lipoproteins characterized by presence of apoprotein B.

## Apolipoproteins

The study of lipoprotein apoproteins in our laboratory is a chapter written simultaneously with the above. It was here, and due to the initiative and hard work of numerous young scientist colleagues, that three of the six well-known apolipoproteins were discovered. And where the primary structure of three of them was first determined. Independently, and simultaneously with my old colleague Havel's laboratory, we also discovered the first demonstrable function of an apolipoprotein. the activation of lipoprotein lipase by apoprotein C-II (115).

During 1966 to 1969, Virgil Brown, newly arrived in the laboratory and essentially beginning his research career, succeeded in isolating and characterizing the VLDL-HDL apoproteins we now know as C-I, C-II, and C-III. This includes identification of the several polymorphic forms of the latter, shown to be due to different amounts of sialic acid (98, 102, 114). The Shores in California had independently also isolated C-III, the most abundant of the C proteins. Several of the terminal amino acids first described required correction (127). This was especially important at that time, because we were promoting the C-terminal nomenclature of this protein, only later yielding to the letter system proposed by Alaupovic, which was clearly better, if imperfect (180). In 1972, C-III was sequenced by Bryan Brewer, Richard Shulman, and other members of the laboratory. Lux, Brewer, Ronan, and John also completed the amino acid sequence and determination of the novel structure of apo A-II in 1972. Apo C-I was sequenced by Shulman, Herbert, et al., in 1975 (175). Apo C-II is the last to have been sequenced, first by Jackson et al., in Houston, then by Herbert, now gone to Providence from Bethesda. Since the

Houston laboratory (established by Gotto and Jackson, when they left Bethesda) also first completed analysis of the sequence of apo-A-I, one can understand a certain proprietary attitude toward primary apolipoprotein structure that was maintained at the Molecular Disease Branch at NHLBI.

As one of the very few laboratories interested in apolipoproteins at the time, we carried out numerous other explorations in addition to those already mentioned. Many dealt with the new C-proteins, including the first inkling that they were exchanging between VLDL and HDL (118). Gotto chose to work for a while with the far less manageable apoprotein B (72, 75, 78, 83, 90, 97). He also took the lead in showing that apo-B was completely missing from plasma in abetalipoproteinemia (121). This was something we had found five years earlier (58); but the sensitivity of techniques had greatly increased and Lees, by now working with Ahrens in New York, had reported that apo-B was present, possibly in an altered antigenic form. The apo-B and apo-C's in lipoproteins from patients with familial hypercholesterolemia also were shown to be the normal (146).

### Triglyceride Hydrolases

One of the richest assets of our clinic was the cohort of patients with a defect we believe should now be called "familial lipoprotein lipase deficiency" (198). The "P family," consisting of three affected siblings, had been acquired as patients by Havel, and he and Gordon had shown that they were missing the "post-heparin clearing factor" in plasma. Ed Korn, whose laboratory I often frequented, had characterized this factor and called it lipoprotein lipase. From the beginning, I nearly always had someone working on "lipase" in the laboratory. It was not a task that everyone enjoyed; the literature on heparin

clearing factor and lipoprotein lipase is enormous, attesting to the difficulties of studying enzyme interactions at oil-water interfaces. To summarize quickly a great deal of work, I should say that we made the following significant contributions to knowledge about triglyceride lipases. First, we worked up a good clinical assay for "post-heparin lipolytic activity" (39), the term I insisted be used then because the heparin-released enzymes were heterogeneous. This old "Ediol" method was used to show that most hyperglyceridemics did not have "PHLA deficiency." It is still in use in a few places. Next we worked very hard to prove that heparin causes at least two lipases to appear temporarily in plasma. The late, versatile Bernard Shore had been the one to show this heterogeneity years earlier. We had to prove all over again to some skeptical journal editors that only one of these enzymes was lipoprotein lipase (shown by Korn and Rodbell to be bound mainly to adipose tissue capillary membranes) and to pinpoint the source of the non-lipoprotein lipase enzyme. LaRosa and Krauss, with the particular help of Greten, Assmann, and Herbert, were principally responsible for showing that the other lipase activity came from the liver (142, 154, 158, 163). This is the enzyme now referred to as (post-heparin) hepatic triglyceride hydrolase. By now it was apparent that all the reports around the world describing measurements of "total PHLA" were worthless. Krauss then took the protamine-sensitivity of lipoprotein lipase--an old observation of Korn's for distinguishing the capillary bound enzymes--and patiently brought it to clinical usefulness (172). Thus we were able to prove for all time that familial lipoprotein-lipase deficiency was a profound deficiency of the one enzyme. The activity of the hepatic lipase was not decreased. It was also shown that patients in the familial type 5 hyperlipoproteinemia group and all the other known mutations

usually had no clear-cut abnormality in activity of either lipase. The discovery of lipoprotein lipase activation by apo-C-II has already been noted (115, 164). Beginning in Sweden and then in other laboratories, including those of Brown and Greten after they left Bethesda for La Jolla and Heidelberg, the lipases have now been separated chromatographically and immunologically. These better techniques will refine the distinctions obtained with the older methods. Perhaps they will also reveal that differences between these enzymes are quite small compared to their functional separation.

The bibliography contains some other results of my having gamboled over broad green fields in the company of so many gifted colleagues, but it's time to close this resumé. There are other papers, some illustrating the ambiguities of a life in research. The discovery of cholesteryl ester storage disease was not related in full for a long time after the first brief notice in an abstract (135, 141, 143); the full pathological description of that extraordinary index case, derived from the attendance of Sloan and myself at the autopsy in another city, is still being written. Multiple observations of patients with different types of Niemann-Pick disease, made with Howard Sloan, still rest unwritten in his records; the oxygenated steryl esters in Wolman's disease that so fascinated Gerd Assmann and me (173) may not be explained for a long time. The little report in which Shulman and I join Bhattacharyya and Connor in describing the third case of  $\beta$ -sitosterolemia and xanthomatosis (183) does not relate how we might have "notched another first" several years earlier had we not had a dispute over interpretation of the mass spectrograph of her plasma. One should always try to be sure. Some credits will be lost, but the universe of knowledge will be the better for caution. The absence of serious retractions is also a measure

of how time was spent in the laboratory. I cannot recall our having printed a grievous error; fortunately we were able to correct most of the small ones ourselves.

The bibliography continues, but circumstances force it to grow more philosophical in its later entries. The trend is probably both natural and irreversible. <sup>5/</sup> Nevertheless, I hope the next papers will reflect some of the joys of the older ones--the hypotheses, the experimental designs, the masses of data, the excruciating first drafts, the bumpy road to the seventh draft, the pain and pleasure of editorial reviews, the apogee of the acceptance and the peaceful denouement spent among the galley proofs. I don't know any other way of life that could have been as good.

Footnotes

- 1/ Prepared as background material for a lecture on the occasion of receiving the Gairdner Foundation Award, November 3, 1978.
- 2/ Zöllner, N., Deutsche Medizinische Wochenschrift, 25 August 1961, No. 34, 1626-1627.
- 3/ A letter from the editor of Science Citation Index in January 1977 (attached) informs me that an article, "Fat Transport in Lipoproteins: an Integrated Approach to Mechanisms and Disorders," was purported to be one of the "500 papers most cited during the years 1961-1975." Largely as a result of this, I seem to have emerged as the most-cited "physiologist" during the period 1961-1976. (Current Contents, 10 July 1978; Science, 20 October 1978.) The chapters in MBID are not counted in this recognition game.
- 4/ In 1968, I resigned as Director of the National Heart Institute, having fulfilled a request of James A. Shannon, Director of NIH, to take the post "for at least one year." I proposed to Shannon that he appoint as my successor Dr. Theodore Cooper, which he did. I returned to the laboratory, now the Molecular Disease Branch, shortly thereafter to become simultaneously Director of Intramural Research, succeeding Berliner. In the fall, Cooper asked me to assemble those experts who believed and those who were skeptical of lipoprotein typing to determine if the methods should be extended extramurally. The conference reached such a consensus and the Lipid

Research Clinic Program was begun. Dr. R. I. Levy was the first chief of the program. At least 8 of the persons who would eventually be directors or co-directors of the first 15 clinics have been trained in the Molecular Disease Branch (Kwiterovich at Johns Hopkins Hospital, LaRosa at George Washington University, Gotto at Houston, Glueck at Cincinnati, Brown at La Jolla, Klimov in Leningrad, Greten in Heidelberg, Eisenberg in Jerusalem).

5/ In July 1975, I assumed the Directorship of NIH.